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Machine Perfusion at 20°C Prevents Ischemic Injury and Reduces Hypoxia-Inducible Factor-1α Expression During Rat Liver Preservation

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Ischemic cholangitis is the main cause of liver failure after transplantation and subnormothermic machine perfusion may represent a better strategy than conventional cold storage, minimizing preservation injury. We compared livers preserved by machine perfusion at 20°C (MP20) or by cold storage at 4°C (CS4) with regard to hypoxia-inducible factor (HIF)-1α mRNA expression and protein stabilization in hypoxic conditions.

Material/Methods: Livers from male Wistar rats were stored on ice at 4°C in UW solution (CS4) or perfused with oxygenated Krebs-Henseleit buffer at 20°C (MP20) for six hours. After preservation, the livers were reperfused for two hours with oxygenated Krebs-Henseleit buffer at 37°C to simulate reimplantation. We collected bile, perfusate, and tissue samples. Transaminases, lactate dehydrogenase, bilirubin, and lactic acid were assayed in the perfusate and bile. ATP/ADP, glycogen, HIF-1α mRNA, and protein expression were measured in the tissue homogenates.

Results: At the end of preservation, as well as after reperfusion, HIF-1α mRNA expression was significantly higher in the ischemic CS4 livers. Although the hypoxic conditions found in CS4 preservation stabilized HIF-1α protein was significantly higher in the CS4 livers at the end of preservation, no difference was observed after reperfusion, likely because of the oxygen in the reperfusion medium. After reperfusion, the MP20 livers released less transaminases and LDH. The MP20 livers had higher ATP/ADP, glycogen, and biliary bilirubin after both preservation and reperfusion when compared with the CS4 livers.

Conclusions: The data demonstrated that MP20 was associated with a lower HIF-1α expression and organ injury with respect to CS4, suggesting that oxygen provided by this preservation setting might approximate the organ request, thus avoiding the ischemic injury usually observed during organ preservation by cold storage.

MeSH Keywords: Cold Ischemia • Cryopreservation • Liver Transplantation

Abbreviations: HIF-1α – hypoxia inducible factor-1α; MP – machine perfusion

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Background

The ischemic period represents an inevitable event during conventional organ preservation before transplantation. The magnitude and severity of reperfusion injury depends on the duration of liver preservation [1].

Nowadays, cold storage is considered the reference procedure for liver preservation, although it triggers events that can result in hepatic ischemia/reperfusion injury. Owing to the growing use of marginal organs that may particularly benefit from optimized preservation techniques, there is a renewed interest in machine perfusion (MP) [2–4]. In particular, hypothermic MP protects livers from non-heart-beating donors (NHBD) and fatty livers during preservation [2,5]. In our previous studies, we showed that steatotic rat livers were better preserved by MP at 20°C (or subnormothermic MP) than conventional cold storage [6,7]. These results have been confirmed [8] and discussed [9] recently. Furthermore, livers from NHBDs also exhibited a marked reduction in damage when preserved by subnormothermic MP, as evaluated as enzyme release, bile flow, and energetic status, which is usually impaired by cold storage [10,11]. This is probably due to the fact that the oxygen conveyed to the liver in subnormothermic MP is similar to the actual hepatic oxygen requirement as highlighted by minimum cell lysis and better liver functionality observed during reperfusion. During machine perfusion at 20°C, hepatic metabolism is not stopped but merely slowed down, probably justifying the prompt recovery observed during reperfusion [7].

Tissue hypoxia induces cell damage, initiating a broad spectrum of adaptive cellular responses by activating transcription factors involved in angiogenesis and glycolysis [12]. Hypoxia inducible factor-1α (HIF-1α) is a regulator of more than a hundred genes. HIF belongs to a large family of proteins and is a heterodimer composed of an alpha (HIF-1α, HIF-2α, or HIF-3α) and a stable, constitutively-expressed beta subunit. Under normoxia, HIFα is hydroxylated by oxygen-dependent prolyl-hydroxylase domain (PHDs)-containing enzymes. Hydroxylated HIFα is recognized by the β-domain of von Hippel-Lindau tumor suppressor protein (pVHL) and subsequently marked for degradation by the 26S proteasome. Prolyl hydroxylation of HIFα is abrogated under hypoxia, allowing HIFα stabilization and accumulation in the nucleus [13]. It is worthy of note that the majority of protein factors involved in glycolysis are upregulated in hypoxia through HIF-1α stabilization [13]. Glycogen utilization is associated with the HIF pathway: recently, the administration of the HIF-1 antagonist induced an increase in hepatic glycogen biosynthesis leading to glycogen accumulation in the liver [14]. Tajima et al. reported that HIF-1α is involved in the regulation of gluconeogenesis and synthesis of glycogen under liver regeneration [15]. To our knowledge, only a few studies have evaluated HIF-1α after cold storage preservation and no evidence about its activation during subnormothermic MP has been reported [16,17].

Advantages of MP have been recognized by recent clinical studies, especially in kidney transplantation [18], although it is not completely clear how this technique supports organ preservation. Based on the aforementioned reports, we designed a study to assess liver injury both at the end of preservation and during the reperfusion period in rat livers preserved by conventional cold storage versus MP at 20°C. We evaluated mRNA and protein expression of HIF-1α, energy status, and antioxidant defense in livers preserved by MP at 20°C or cold storage.

Material and Methods

Chemicals

All chemicals were purchased from Sigma (Milan, Italy).

Animals and surgery

Male Wistar rats (Harlan-Nossan, Italy), weighing 250 to 300 g, were housed in groups of four and allowed free access to food and water until sacrificed. Animals in this experimental study were cared for according to a protocol approved by the University Commission for Animal Care and the Italian Ministry of Health, according to the provisions of the EU Directive 2010/63/EU for animal experiments. Animals were divided in two independent sets of experiments. In the first setting, experiments were stopped at the end of six hour preservation by MP at 20°C (MP20, n=8) or by conventional cold storage at 4°C (CS4, n=8). In the second setting, experiments were stopped at the end of two hours reperfusion at 37°C, for both livers preserved by MP at 20°C (MP20-R, n=8) and livers preserved by conventional cold storage (CS4-R, n=8). Tissue and serum samples from control, non-perfused livers (n=6, C) were also collected. Livers were isolated as previously described [19,20]. Briefly, rats were anesthetized with 40 mg/kg sodium pentobarbital given intraperitoneally, the abdomen was opened and 500 units of heparin were administered via the inferior vena cava; bile duct was cannulated with polyethylene tubing and the portal vein was cannulated with a 16 G catheter (Johnson and Johnson, Arlington, UK). After flushing with Ringer lactate, livers were preserved for six hours by cold storage or MP at 20°C.

Machine perfusion preservation

Livers preserved by MP were placed in a jacketed organ chamber and connected to a recirculating perfusion system. Livers were perfused with oxygenated KH solution containing: (in mmol/L) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.25 $CaCl_2$, 25 $NaHCO_3$, 20 N-(2-hydroxyethyl)-piperazine-N-(2-ethanesulfonic

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acid) (pH 7.4), 5 glucose, and 5 n-acetylcysteine (NAC) (pH 7.4, 20°C) [7]. A PO_2 of about 700 mBar at 20°C was measured in the perfusate by a glass oxygenator. A water column connected to the portal vein was used to measure the portal pressure; before each experiment, a precalibration of the water column was performed. Starting perfusion pressure was about 6–7 mm Hg [21,22].

Cold storage preservation

Livers were flushed *in situ* with ice-cold UW for two minutes (50 mL) and maintained at 4°C in this solution for six hours [7].

Normothermic reperfusion

During normothermic reperfusion livers were perfused with oxygenated KH solution at 37°C for two hours. An identical set-up was applied to livers preserved by MP or cold storage, to simulate organ reperfusion after implantation in the host.

Assays

Hepatic injury was assessed by release into the effluent solution of aspartate transaminase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), determined by an automated Hitachi 747 analyzer (Roche/Hitachi, Indianapolis, IN, USA). Total bile production was measured during MP and reperfusion periods; bilirubin and LDH levels in bile were determined by an automated Hitachi 747 analyzer. At the end of both the preservation and reperfusion periods, tissue samples were quickly removed and frozen in liquid nitrogen. Tissue ATP was measured by the luciferin-luciferase method using the ATPlite luminescence assay kit (Perkin Elmer Inc., USA) following the manufacturer's instructions with some modifications. Briefly, frozen tissue was homogenized in TCA 30% (100 mg/mL) and centrifuged at 3,000 g ×15 minutes. The supernatant was diluted in a 100 mM phosphate buffer at pH 7.75 and finally the sample was assayed. Tissue ADP was assayed as follows: ADP was converted into ATP using the pyruvate kinase reaction by adding 1.5 mL of the diluted sample to 0.5 mL of a buffer containing 40 U/mL pyruvate kinase, 40 µM phosphoenolpyruvate, 10 mM KCl, and 40 mM $MgSO_4$ in 40 mM tricine buffer, pH 7.75. ADP was calculated by subtracting the ATP values previously obtained [23]. The glycogen assay was performed as described by Bennett et al. [24] with some modifications. Briefly, liver samples were homogenized in a solution of 10% $HClO_4$ and centrifuged at 280 g for 15 minutes. The pellets were resuspended with 2 mL of deionized H_2O. Then 0.1 mL of sample was mixed with 0.2 mL of 5% phenol and 1 mL of H_2SO_4. After 30 minutes, samples were measured at 490 nm. The hepatic concentration of total glutathione was measured at the end of preservation and reperfusion by a GSH assay kit (Cayman, Ann Arbor, MI, USA).

Table 1. List of forward and reverse primers used in experiments.

Gene	Sequence
Rat HIF-1α	Forward 5'-ACA AGA AAC CGC CTA TGA CG-3' Reverse 3'-TAA ATT GAA CGG CCC AAA AG-5'
Rat Ubiquitin C	Forward 5'-CAC CAA GAA CGT CAA ACA GGA A-3' Reverse 3'-AAG ACA CCT CCC CAT CAA ACC-5'
Rat Tubulin	Forward 5'-AGA AGC AAC ACC TCC TCC TCG-3' Reverse 3'-ATA CAC TCA CGC ATG GTT GCT G-5'

GSSG was determined after derivatization of GSH with 2-vinylpyridine [25]. Protein concentration was measured by Lowry protein assay [26].

HIF-1α mRNA was analyzed using real-time polymerase chain reaction (RT-PCR), performed as previously described [27]. Briefly, total RNA was isolated from liver samples with TRI reagent (Sigma-Aldrich) [28]. The cDNA was generated using iScript Supermix (BioRad) and amplified using Platinum Sybr Green qPCR mix UDG; forward/revers primers for HIF-1α, ubiquitin (UBC), and tubulin (TUB) were synthesized by Eurofins (Vimodrone, MI, Italy) (Table 1). The efficiencies of gene amplification of HIF-1α, UBC, and TUB (respectively 108.8%, 98.6%, and 102.4%), were established by means of calibration curves in a cDNA concentration range of 10–0.1 ng/µL. In the Master Mix, final concentrations of primers and samples were, respectively, 500 pmol/mL and 0.125 ng/mL. The expression of the housekeeping genes remained constant in the considered experimental group. Ubiquitin and tubulin were used as reference genes. The amplification was performed in an ABI prism 7000 (Applied Biosystems, Deutschland Inc., Darmstadt, Germany) through two-step cycling (95–60°C) for 45 cycles. All samples were assayed in triplicate. The results were normalized to the housekeeping genes, fold change of the gene expression was calculated by the ΔCt method, and comparison between groups was calculated using the ΔΔCt method.

The HIF-1α protein expression was analyzed on the nuclear fraction of tissue homogenates with a HIF-1α ELISA kit (HIF-1α Transcription Factor Assay Kit, Cayman, USA), following the manufacturer's instructions. The nuclear fraction was purified from homogenates with the Nuclear Extraction Kit (Cayman, USA). Total protein content in the nuclear extracts was assayed as described previously [29].

Statistical analysis

Data are presented as the mean ± standard errors (SE). Statistical analysis for multiple comparisons of normally distributed data was performed by the one-way ANOVA test; Tukey test was used for post hoc analysis. Analysis of correlation was performed by fitting linear models in R (R Statistical

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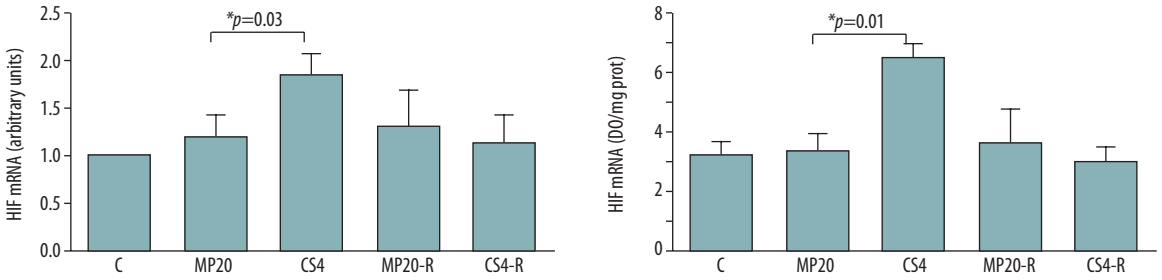

Figure 1. HIF-1α mRNA and protein expression at the end of preservation and after reperfusion. The MP20, MP20-R, and CS4-R groups showed no significant differences in HIF-1α mRNA and protein expression with respect to the control animals; CS4 livers showed an mRNA and protein expression significantly higher with respect to MP20. C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

Software, v.3.4.0); the significance of correlation was calculated by Pearson's product-moment correlation and Spearman's rank correlation rho.

Results

HIF-1α mRNA and protein expression

Hepatic HIF-1α mRNA showed a significant increase only at the end of cold storage preservation in comparison with the other groups considered (Figure 1). Of note is the fact that no increase in HIF-1α mRNA was found in MP20 and MP20-R groups as compared with livers from the control group (Figure 1).

The HIF-1α protein expression showed a similar trend to HIF-1α mRNA for all the groups considered (Figure 1). Interestingly, comparable results of HIF protein expression were found in MP20 and MP20-R versus the control group (Figure 1).

Hepatocellular injury at the end of preservation using MP at 20°C

Enzyme levels were measured to estimate liver injury; very low levels of AST, ALT, and LDH were found after six hours of preservation in MP20 (in mU/mL, AST 13.5±1.1; ALT 4.9±0.9, and LDH 0.93±0.09).

Bile production did not stop in MP20: the cumulative bile was 29±5 µL/g and the bile flow was continuous and stable during the six hours of organ preservation (0.084±0.01 µL/min/g).

Hepatocellular injury during reperfusion

The parenchymal integrity was compromised in livers from CS4-R rats, as showed by the significant release of the AST, ALT, and LDH during normothermic reperfusion (Figure 2). Contrarily, the enzyme release during reperfusion was reduced in MP20-R livers when compared to CS4-R livers (Figure 2).

We found a very strong positive correlation (R^2=0.78) when pairing tissue HIF-1α protein expression at the end of preservation with AST release at the end of reperfusion for each sample (Figure 3). Furthermore, the correlation was highly significant using both Pearson's and Spearman's tests (p=4.51×10^{-8} and 3.70×10^{-6}, respectively).

No significant difference in bile flow was observed during reperfusion in CS4-R with respect to MP20-R: cumulative bile was 50±7 µL/g and 48±9 µL/g, respectively. Interestingly, an increase in biliary bilirubin output and lower levels of biliary LDH enzymes were detected during reperfusion in MP20-R as compared with CS4-R (Figure 3). A seven-fold increase in biliary bilirubin was detected in MP20-R as compared with CS4-R organs (Figure 4).

Energy status

We evaluated the energy status in hepatic tissue collected at the end of the preservation: a marked decrease in ATP content was observed in preserved livers as compared with the control, not-preserved, group (Figure 5). Interestingly, the ATP levels and the ATP/ADP ratio were higher in MP20 livers as compared with CS4 livers (Figure 4). The MP exerts a positive effect on the hepatic ATP content as supported by the continuous bile flow, known as an energy-dependent process and which was observed during preservation with MP at 20°C [10]. The same trend was found for ATP content and the ATP/ADP ratio at the end of reperfusion in MP20-R livers and CS4-R livers (Figure 5).

No differences in hepatic glycogen levels were observed between CS4 and MP20 livers at the end of preservation (Figure 6).

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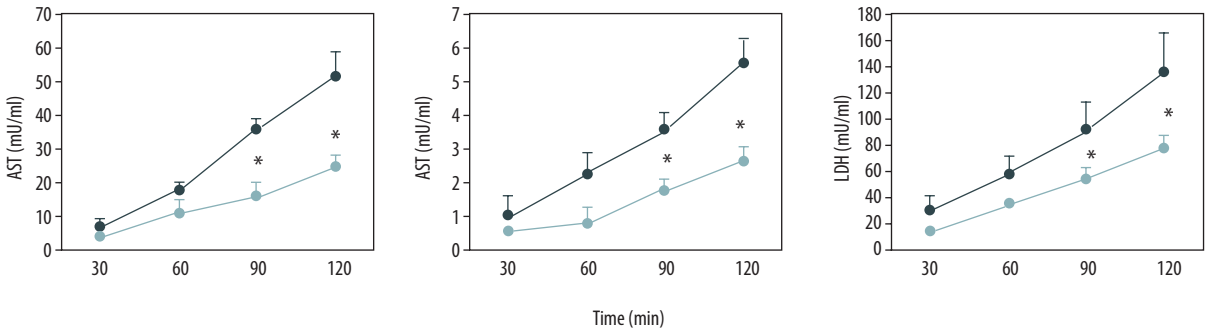

Figure 2. AST, ALT, and LDH released into the perfusate during reperfusion. Levels of hepatic enzymes (AST, ALT, and LDH) released by CS4-R livers (black circles) were significantly higher than those released by MP20-R livers (white circles) (* $p \leq 0.05$). C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

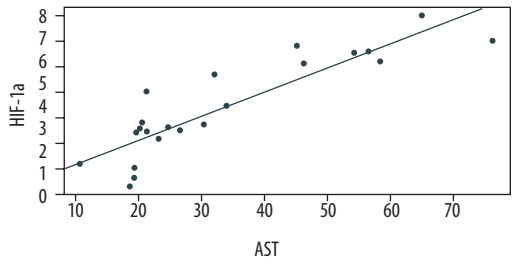

Figure 3. Correlation between HIF-1α hepatic protein expression at the end of six hour preservation and AST release at the end of reperfusion. C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

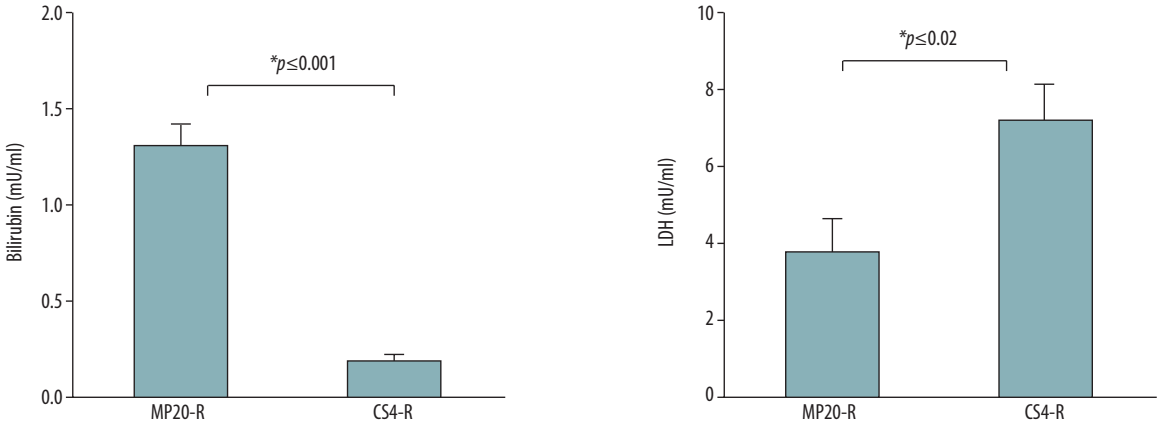

Figure 4. Bilirubin levels and LDH release into the bile at the end of reperfusion. Higher levels of bilirubin and lower LDH release were detected in the bile of MP20-R livers with respect to CS4-R livers. C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

Of note, higher levels of glycogen were found after reperfusion in MP20-R organs, when compared with CS4-R organs (Figure 6).

Reduced and oxidized glutathione (GSH and GSSG) levels

Hepatic glutathione plays a central role in the defense of cells against free radical (ROS) injury [30]. We evaluated GSH/GSSG at the end of preservation and reperfusion. A marked decrease

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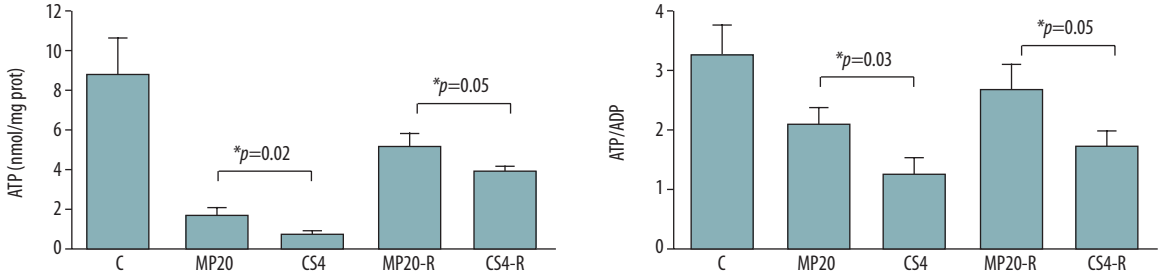

Figure 5. ATP levels and ATP/ADP ratios at the end of preservation and reperfusion periods. ATP levels and ATP/ADP ratios were significantly lower in CS4 and CS4-R liver with respect to MP20 and MP20-R livers, respectively. C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

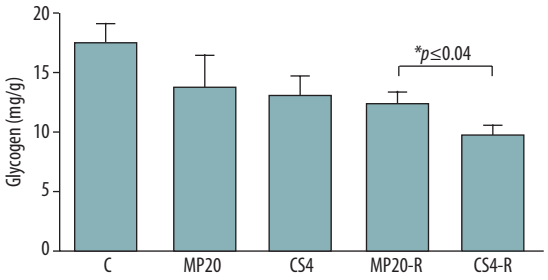

Figure 6. Glycogen content at the end of both the preservation and reperfusion periods. At the end of normothermic reperfusion, a decrease in glycogen stores was observed in CS4-R livers as compared with MP20-R livers. C – not preserved controls; MP20 – six hours machine perfusion at 20°C; CS4 – six hours cold storage; MP20-R – six hours machine perfusion at 20°C plus two hours of normothermic reperfusion; CS4-R – six hours cold storage plus two hours of normothermic reperfusion.

in the GSH/GSSG ratio was observed in both CS4 and MP20 livers as compared with the control group (7.2±0.9 and 8.4±1.1 in CS4 and MP20, respectively, versus 15.5±2 in control livers). No difference in the GSH, GSSG and GSH/GSSG ratio was found in MP20-R livers as compared with CS4-R livers (9.9±1.8 and 11.7±2.1, respectively).

Discussion

These results showed that the limited injury observed in liver preserved by MP at 20°C was associated with a reduction in HIF-1α mRNA and protein expression as compared with organs preserved by cold storage; the HIF-1α levels in MP 20°C preserved livers are comparable with those observed in control (not-preserved) organs. The difference between groups was evident only at the end of preservation and disappeared after oxygenated reperfusion, probably because of the activation of oxygen-dependent HIF-1α prolyl hydroxylases. Furthermore, a strong positive correlation was been observed between the concentration of active HIF-1α at the end of organ preservation and AST levels at the end of reperfusion, suggesting that a proper oxygen delivery to the organ may improve liver conditions after reperfusion.

Energy status

It is well known that a decrease in hepatic ATP occurs in ischemic livers, phosphorylation being seriously affected during ischemia: when the liver is placed in a hypothermic solution during cold storage, concomitant ATP deletion leads to failure in cellular homeostasis [31,32]. In the present study, both MP at 20°C and cold stored livers showed lower ATP levels and ATP/ADP ratio, compared with the control. Interestingly, hepatic ATP was about twice as much in the MP20 livers when compared with the CS4 group at the end of the preservation period. These findings support the higher energy status observed after reperfusion: ATP/ADP was higher in MP20-R with respect to CS4-R, suggesting a greater capacity to resume functionality after transplantation [10]. The maintenance of cellular energy status is important for liver preservation and a low ATP/ADP ratio is a central issue limiting preservation quality. Bile flow has also been employed as an indicator of hepatic IR damage and offers the possibility of eliminating toxic metabolic products during preservation [33,34]. The present study results indicated increased biliary excretion of bilirubin associated with a decrease in biliary levels of LDH in MP 20°C preserved livers. We previously proposed that MP performed at 20°C protects both hepatocytes and cholangiocytes [20] and this hypothesis was supported by work that showed the liver's ability to eliminate metabolic products such as bilirubin probably associated to hepatic ATP levels [35]. Interestingly, Tolboom et al. recently found an increase in serum bilirubin

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in transplanted livers obtained from NHBDs, especially in livers preserved by MP at 20°C [36].

The improved recovery of energy status during reperfusion of MP 20°C stored livers was strictly connected with the higher ATP/ADP levels at the end of preservation and the significant high glycogen content after reperfusion. No significant difference in the tissue levels of glycogen was observed between MP and cold storage livers after six-hours of preservation. We posit that during MP 20°C preservation the aerobic conditions associated with an adequate supply of substrates for glycolysis, such as glucose, reduces the need to breakdown glycogen stores and that in hypothermic preservation a marked reduction of ATP hydrolysis avoids the need to use glycogen especially after six hours.

Perfusions of livers and hypothermia have been associated with loss of sinusoidal endothelial cells (SECs). Some studies have suggested that SECs undergoes apoptosis after cold ischemia and reperfusion of the liver: this phenomenon is critical in the graft failure following transplantation [37]. Other data, in which UW is used as a preservation solution, have demonstrated that early SEC necrosis does not appear to be of major importance in the graft failure mechanism [38]. A limitation of this study is that the reperfusion performed without whole blood may mask the loss of sinusoidal endothelial cells at reperfusion in a transplant model.

We previously reported that marginal organs preserved by MP at 20°C provided a grade of preservation/reperfusion injury similar to that observed in normal livers preserved by cold storage [7,10]. The limited damage in marginal livers could be explained with the absence of ischemia observed during MP at 20°C preservation: the oxygen supply is sufficient for mitochondrial activity at 20°C even in marginal livers as confirmed by the higher ATP content using MP versus cold storage [10]. The successful transplantation of marginal organs, such as ischemic livers, preserved with MP at both 20°C and 30°C when compared with conventional cold storage, confirms and supports the superiority of this new preservation technique as well as innovations in the temperature used [39]. The present work provides an explanation for these surprising results. Recently, we also reported that subnormothermic MP was able to resuscitate liver grafts from large NHBD animals [11].

Induction and stabilization of HIF-1α

The absence of HIF-1α induction in livers preserved with MP at 20°C was the main finding in this study representing the crucial cellular response strictly connected with higher hepatic tolerance to preservation injury as compared with that found in cold-stored livers. Recent progress has clearly demonstrated that low oxygen influences gene expression through activation of hypoxia-inducible transcription factors (HIFs) [39]. It is known that HIF-1α regulates the adaptation response of organs to the changes in oxygenation levels: cold ischemia preservation is characterized by a lack of oxygen supply to the liver, but only a few studies have quantified the hepatic levels of HIF-1α. In this study, six-hour cold stored livers showed an increase in the HIF-1α mRNA and protein expression, supporting previous published data [16]. On the contrary, MP at 20°C, performed under our experimental setting, showed a very limited HIF-1α mRNA and protein expression. In literature, it has been reported that an estimated hepatic oxygen requirement for rat liver at 20°C is about 0.8 µmol/min/g [40]; in our experimental setting of rat livers at MP at 20°C, the oxygen supply reached 1.4 µmol/min/g, suggesting that, if the estimates are correct, oxygen supply meets liver requirement. Recent studies concluded that ROS production did not have a significant role in HIF-1α protein stabilization during hypoxia and that mitochondria can modulate cellular hypoxic response through altered respiratory activity [41]. The tripeptide GSH, the major antioxidant and redox regulator in cells, is important in combating oxidation of cellular constituents. In our study, we evaluated the GSH/GSSG ratio of liver at the end of preservation: the comparable results obtained in MP and cold storage preserved livers allowed us to conclude that in our model, too, the HIF-1α stabilization, observed after conventional cold storage, was probably independent of ROS production. Zaouali et al. have shown that after 24-hour cold storage, the restoration of oxygen during reperfusion induces a decrease in HIF-1α expression [16]; for this reason and owing to the relatively short period of cold storage used in our study, the HIF-1α level did not differ in livers preserved with MP or cold storage after the reperfusion period. Otherwise, our data showed an increase in HIF-1α after six-hour of cold storage preservation.

The central goal of organ preservation is to deliver a viable graft with a minimum risk of postoperative organ dysfunction. The current clinical practice of liver preservation is based on cold storage preservation in which the anaerobic metabolism induces a depletion of energy stores and an increase in metabolic substrates. On the contrary, MP preservation performed at subnormothermic temperature avoids cold injury, ischemia/reperfusion damage, and accumulation of metabolic products. We are aware of the limitations of the rat model of liver preservation injury; however, it should be considered that this model has provided great contributions to the development of optimal preservation solutions [42,43] and is currently used for the investigation of new preservation techniques [44].

Conclusions

The present work showed that reduced HIF-1α expression was associated with the protection of livers preserved by MP at

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20°C, highlighting how this technique performed at subnormothermic temperature has the concrete potential of avoiding ischemic insult, the latter being the real culprit with regard to preservation injuries observed in cold storage transplanted organs. The subnormothermic temperature used during MP preservation may also favor the successful translation of this technique from experimental studies into clinical practice.

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